

## RESEARCH ARTICLE

# Red Ginger (*Zingiber officinale* var. *Amarum*) Infusion Increases Concentration of Luteinizing Hormone and Testosterone in Male Rats

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## ABSTRAK

**Pendahuluan :** Pengobatan dengan tanaman herbal banyak dikembangkan sebagai pengobatan komplementer untuk meningkatkan hormon seksual. Pemberian jahe merah dengan pelarut etanol terbukti meningkatkan sel spermatogenik, motilitas dan morfologi spermatozoa pada tikus. Namun efek jahe merah dengan teknik infusasi belum pernah dilakukan. **Tujuan:** Mengetahui pengaruh infusa jahe merah terhadap kadar LH dan testosteron tikus Wistar jantan.

**Metode:** Penelitian ini adalah penelitian eksperimental dengan rancangan *Post Test Only Control Group Design*. Sampel tikus wistar jantan usia + 3 - 4 bulan, BB : 200-250 gram sebanyak 24 ekor, dibagi 4 kelompok. Kelompok I (G-1) kelompok kontrol diberi aquadest 2 ml/hari. Kelompok 2 (G-2), kelompok 3 (G-3), dan kelompok 4 (G-4) merupakan kelompok perlakuan masing-masing diberi infusa jahe merah dengan dosis 10%, 20%, dan 40% yang dilarutkan dalam 2 ml aquadest. Hari ke 15 dilakukan pengambilan darah untuk pemeriksaan LH dan testosteron.

**Hasil:** Uji *One Way Anova*, menunjukkan terdapat perbedaan kadar LH dan testosteron di antara kelompok,  $p < 0,001$ . Hasil *Post hoc LSD test* menunjukkan bahwa kadar LH (0.350) pada G-1 lebih rendah dibanding G-2 (1.082), G-3 (1.253), dan G-4 (0.923),  $p < 0.005$ . Sedangkan kadar testosteron pada G-1 (0.2718) lebih rendah dibanding G-2 (0.7797), G-3 (0.8285), dan G-4 (0.9142),  $p < 0.005$ .

**Kesimpulan:** Pemberian infus jahe merah selama 14 hari dapat meningkatkan kadar LH dan hormon testosteron tikus wistar jantan.

**Kata kunci :** LH, Testosteron, Infusa Jahe Merah

## ABSTRACT

**Introduction:** Herbal remedies have been developed as a complementary therapy to increase sex hormones. The administration of the ethanolic extract of red ginger (*Zingiber officinale* var. *Amarum*) has been shown to increase spermatogenic cells, motility and morphology of sperm in mice. However, the effect of red ginger infusion on the level of testosterone and luteinizing hormone (LH) has not been done. **Objective:** to examine the effect of red ginger infusion on LH and testosterone levels of male Wistar rats.

**Methods:** The study was designed as an experimental post-test only control group design. Twenty four male Wistar rats of 200-250 g body weight aged + 3-4 months were divided into four groups. a control group (G-1) was given distilled water 2 ml/day. Group 2 (G-2), Group 3 (G-3), and group 4 (G-4) were treated with 10%, 20%, and 40% infusion by dissolving 2 ml of ginger in distilled water. On day 15, blood samples were taken and assayed for LH and testosterone.

**Results:** The one-way ANOVA test showed difference in levels of LH and testosterone between groups ( $p < 0.001$ ). Post Hoc LSD test results showed that the levels of LH of G-1 (0.350) was significantly lower than that of G-2 (1.082), G-3 (1.253), and G-4 (0.923),  $p < 0.005$ . While the levels of testosterone in the G-1 (0.2718) significantly lower than the G-2 (0.7797), G-3 (0.8285), and the G-4 (0.9142) ( $p < 0.005$ ).

**Conclusion:** red ginger infusion for 14 days can increase the levels of LH and testosterone in male rats.

**Keywords:** LH, Testosterone, infusion of Red Ginger

## INTRODUCTION

The aging process is one of the problems that can not be avoided by both men and women. Aging in men is accompanied by a decrease in testosterone levels with different symptoms and signs known as andropause or late onset of hypogonadism (LOH) (Zen, 2009). Decreased testosterone production by the Leydig cells stats in middle (between ages of 45-59 years) and

are associated with increased secretion of luteinizing hormone (LH). It is associated with reduced Leydig cell number in testis or LH receptors on Leydig cells leading to that the hypothalamic-pituitary-testicular axis does not work optimally (Pangkahila, W. 2007). Red ginger (*Zingiber officinale* var. *Amarum*) is one of medicinal plants commonly used by to increase sexual performance and fertility. Subjects consuming

brewed red ginger have been reported to have increased sexual appeal and erection (Rudy S, 2013). The study findings reported by Mohammad (2010) showed that the ethanolic extract of red ginger extract have a positive effect on sperm quality (concentration, motility, morphology, and testosterone. However, the effect of water infusion red ginger on testosterone and LH levels has not been studied.

Pure water is neutral, with a pH of 7.0 at 25 °C considered as solvent with potential benefits. Infusion technique has several advantages when compared with other extraction techniques. besides practical, infusion be executed in a way that is simpler, faster, and cheap.

Red ginger (*Zingiber officinale* var. *Amarum*) has an active substance including arginine, non-essential amino acid playing a role in the immune system, cellular immunity, the formation of spermatozoa stimulant on of the pituitary follicle-stimulating hormone (FSH) and LH (Srivastava S 2006). In addition, the active substances in red ginger has a molecular structure similar to that of weak androgenic steroid (i.e., beta sitosterol). Beta sitosterol can act as a androgen precursor that can be converted into testosterone in peripheral tissues by various enzymes. In addition, they can also directly affect the level of microsomal enzymes in the Leydig cells stimulating the synthesis of testosterone (Jones, T., H. 2008). Maisuri, RA showed that beta sitosterol has been shown to increase spermatogenesis, the activity of Leydig cells and testosterone (Maisuri, RA. 2013). This present study aimed to determine the effect of infusion red ginger (*Zingiber officinale* var. *Amarum*) infusion on levels of luteinizing hormone (LH) and testosterone in male Wistar rats (*Rattus norvegicus*).

## METHODS

The study was designed as an experimental post-test only control group. Twenty male Wistar rats aged + 3-4 months, weighing 200-250 grams were divided into 4 groups: Group 1 (G-1) as control given aquadest 2 ml/ day, Group 2 (G-2), Group 3 (G-3), and group 4 (G-4) treated with infusion of red ginger 10%, 20%, and 40% respectively by dissolving in 2 ml of distilled water per day. The treatments were given for 14 days. On day 15 blood samples were taken through medial canthus of the orbit approximately 1 - 1.5 ml by using a micro-hematocrit capillary tubes. Before the analysis, the blood samples were processed in the clinical laboratory, where the blood samples placed onto Eppendorf, then centrifuged, the serum was taken and stored in eppendorf at -80 °C.

## Testosterone Assay

Levels of testosterone were determined with indirect enzyme-linked immunosorbent assay (ELISA) assay (spectrophotometrically determined at  $\lambda = 450$  nm). The number of testosterone that is bound to plasma proteins albumin and SHBG plus free testosterone that is not bound to plasma proteins were calculated. First, a number of microtiter well were placed on the holder. Each 25  $\mu$ L standards, controls, and samples were distributed using a new tip can be disposed on wells that have been adjusted. Then 200  $\mu$ L Enzyme Conjugate distributed to each well and mixed thoroughly in 10 seconds. Then incubated for 60 min at room temperature (without covering the plate). After that whipped and issued for the contents of the well and rinse wells 3 times using liquid diluted Wash (400  $\mu$ L per well). The well was then cleaned with absorbent paper to remove residual droplets. 200  $\mu$ L substrate fluid was added sin each well and incubated for 15 min at room temperature. The enzymatic reaction was stopped by adding 100  $\mu$ L stop fluid in each well. Then the optical density (OD) was determined in each well at  $450 \pm 10$  nm with a microtiter plate reader, read within 10 minutes after administration of the liquid stop. Testosterone levels were expressed in units of ng/dl serum.

## LH Examination

Serum LH levels were evaluated by ELISA using a spectrophotometer at  $\lambda$  450 nm. LH levels were assessed in mIU/ml. First, 100  $\mu$ L of standards, blanks, or the sample to the bottom of the well micro-ELISA plate, without touching the walls or creating foam. Then stirred gently and closed with a seal that has been provided. Incubation was then carried out for 90 minutes at 37 °C. Fluid in each well was cleaned but not washed, and then immediately added 100  $\mu$ L Detection Ab Biotinylated fluid in each well and a cap with a seal. Plate tapped gently to ensure thorough mixed and incubated for 1 hour at 37 °C. Then aspiration on each well and washed three times. Washing was performed by filling each well Wash Buffer (+ 350  $\mu$ L). After washing, the remaining wash buffer was cleaned by sucking or pouring. The plate is reversed and patted the thick paper and clean absorbent. Then 100  $\mu$ L HRP Conjugate fluids were added in each well, then covered with a seal and incubated for 30 min at 37 °C. Then the washing process was repeated five times. 90  $\mu$ L liquid and the substrate was added to each well, covered with a new seal plate and incubated about 15 minutes at 37 °C and protected from light. The reaction time can be shortened or elongated by a distinct change in color occurs, but no more than 30 minutes. When the color

Table 1. LH and testosterone level mean of Male Wistar rats

variables	Groups				P Anova
	G-1 (N = 6; $\pm$ SD)	G-2 (N = 6; $\pm$ SD)	G-3 (N = 6; $\pm$ SD)	G-4 (N = 6; $\pm$ SD)	
LH (mIU / mL)	0.350 ( $\pm$ 0.085)	1.082 ( $\pm$ 0.236)	1,253 ( $\pm$ 0.153)	0.923 ( $\pm$ 0.359)	0000
Testosterone levels (mIU / mL)	0.2718 ( $\pm$ 0.089)	0.7797 ( $\pm$ 0.193)	0.8385 ( $\pm$ 0.076)	0.9142 ( $\pm$ 0.2881)	0000

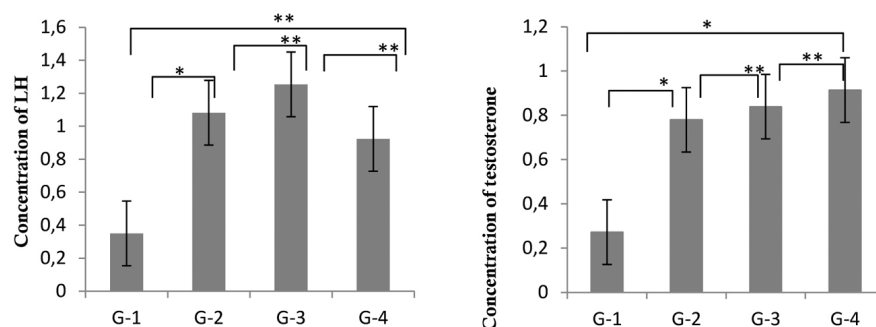


Figure 1. Concentration of LH and Testosterone: \*  $p < 0.001$ ; \*\*  $p > 0.05$

changes evident in the standard wells, the reaction should be terminated, and 50 $\mu$ L stop fluid was added to each well. The sequence for adding liquid stop should be equal to the order of the substrate liquid. Then the optical density (OD) was determined on each well at a time, using a micro-plate reader set to 450nm.

### Statistical Analysis

The data were analyzed using computer software. Since the data obtained has a normal and homogeneous distribution, one way ANOVA was followed by Post Hoc LSD test. Statistical analysis was considered significant when  $P < 0.05$ . This study was conducted after obtaining ethical approval from the ethics committee of Medical Faculty of UNISSULA Semarang.

### RESULTS

The mean LH and testosterone level after the red ginger infusion for 14 days the is presented in Table 1.

Table 1 shows that the highest level of LH was found in G-3 (1.253;  $\pm$  0.153), followed by the G-2 (1.082;  $\pm$  0.236), G-4 (0.923;  $\pm$  0.359), and G-1 (0.350;  $\pm$  0.085). the highest testosterone levels was found in the G-4 (0.9142;  $\pm$  0.281), followed by the G-3 (0.8285;  $\pm$  0.076), G-2 (0.7797;  $\pm$  0.193), and G-1 (0.2718;  $\pm$  0.089).

The data normality and homogeneity test of LH levels in all groups showed normal distribution and homogeneous ( $p > 0.05$ ). Thus the statistical test used

was the one way ANOVA followed by Post Hoc LSD test. ANOVA test results showed that there was a difference in levels of LH between groups ( $p < 0.001$ ). Similarly, the results of ANOVA analysis on testosterone levels also showed a significant difference ( $p < 0.001$ ).

LSD Post Hoc Analysis showed that the level of LH in the G-2, G-3 and G-4 was significantly higher than that of G-1 ( $p < 0.001$ ). While the levels of LH in the G-4 was lower than that of G-3 and G-2, but statistically not significant ( $p > 0.05$ ). Similarly, the level of testosterone in the G-2, G-3 and G-4 was significantly higher than that of G-1. While the levels of testosterone in the G-4 was higher than that of G-2 and G-3, but not statistically significant ( $p > 0.05$  (figure 1).

### DISCUSSION

The results of this study showed that the infusion of the red ginger (*Zingiber officinale* var. *Amarum*) infusion with concentrations of 10%, 20%, and 40% was shown to increase levels of luteinizing hormone (LH) and testosterone on male Wistar rats (*Rattus norvegicus*). It is important to note that the infusion of red ginger at the concentration of 20% can increase levels of the testosterone and LH. But the administration of red ginger at the concentration of 40% increased levels of testosterone and decreased levels of LH. This suggest that elevated levels of LH at the concentration of 20% red ginger can stimulation of LH on Leydig cells to secrete testosterone. On the other hand decreased levels of LH and increase testosterone levels red



ginger infusion at the concentration of 40% leads to testosterone negative feedback on LH secretion. This is consistent with the finding of the previous studies that steeper when testosterone levels in peripheral blood circulation will cause negative feedback to the pituitary thus reducing the synthesis and secretion of LH by the anterior pituitary (Sherwood, L. 2007). Referring to the results of this study, the compounds contained in red ginger infusion can be hypothesized as a stimulator of the anterior hypofysis which then stimulate the Leydig cells to produce testosterone.

The finding of the present study supports a study conducted by Mohammad Hefni in 2010 on the effect of red ginger (*Zingiber officinale* Rosc) extract on the quality of sperm in rats (*Rattus norvegicus*) exposed to allethrin. Allethrin causes histological changes in the testis characterized by damage of the mitochondrial membrane of Leydig cells leading to the steroidogenesis resulting in decreased production of the testosterone hormone. As a result of impaired spermatogenesis process and causes a decrease in sperm quality (Zhang *et al.*, 2007). The results showed that the red ginger extract at the dose of 200 mg/kg can improve sperm quality (concentration, motility, viability) and able to reduce the number of abnormal spermatozoa. Evidence suggests that the quality (concentration, motility, and morphology) spermatozoa are more influenced by endogenous testosterone levels rather than exogenous testosterone. Endogenous testosterone serves to control the meiotic division during spermatogenesis capable of improving the quality of spermatogenesis and sperm produced (Zhang *et al.*, 2007). Furthermore, administration of exogenous testosterone can cause infertility due to negative feedback on the anterior pituitary leading to decreased LH and FSH (Erstad, S. and Havens, L., 2006). In addition, red ginger also has been shown to improve the transport of cholesterol to be converted to pregnenolone by the enzyme of cytochrome P450 in Leydig cell mitochondrial membrane that can be converted into testosterone (Zhang *et al.*, 2007). Evidence shows that transport cholesterol from the membrane to the outer membrane of mitochondria is carried by steroidogenic acute regulatory protein (StAR), a mitochondrial protein with a size of 30 kDa contained in adrenal and gonadal corteks. StAR has an important role in testosterone synthesis. Therefore, individuals with XY chromosome mutated gene STAR have androgen deficiency produced by Leydig cells and is characterized by the typical female genital organs (Papadopoulos V, Miller, 2012). However, whether the administration of red ginger is able to increase the concentration of StAR transport in mitochondria

requires further studies.

Another study conducted by Mahendra in 2009 showed that the extract of red ginger (*Zingiber officinale* roscoe var. *Rubrum*) at the dose of 600 mg /kg for 50 days increased concentrations of, motility and sperm count in male rat (*Rattus norvegicus*). This is caused by the arginine contained in ginger. Arginine is a non-essential amino acid playing a role in the cellular immune system and sperm formation (spermatogenesis). Arginine serve as a precursor of molecule NO (Nitrogen Oxide) which generates a signal between cells to the metabolism. Conversion of arginine to NO is catalyzed by the enzyme NO synthase (NOS) contained in the sperm cell cytosol (Srivastava *et al.*, 2006). Nitric Oxide plays a role in spermatogenesis by stimulating the production of testosterone, activating the guanylate cyclase enzyme. According to Srivastava (2006) NO acts as an activator of guanylate cyclase, the enzyme guanylate cyclase which will increase the formation of intracellular Cyclic guanosine monophosphate (cGMP). NO interaction with the receptor on the cell wall spermatozoa causing an enzyme guanylate cyclase of the cell wall into the cytoplasm. The increase in the enzyme guanylate cyclase causes an increase in intracellular cGMP. The formation of intracellular cGMP activates "protein kinase" and "ion signal" that would affect the cell nucleus so that a variety of genes that regulate the biosynthesis of testosterone become active and start testosterone synthesis (Kaspul, 2007).

Testosterone also plays a role in the process of sperm maturation in the epididymis since its function is highly dependent on the hormone testosterone. Important compounds that support the process of maturation in the epididymis includes ion (Ca, Na, K and Cl); substrates (protein, sialic acid, glycogen, lactic acid, phospholipids); and enzymes (LDH, acid phosphatase, and alkaline phosphatase). When epididymis testosterone deficiency will cause malfunctioning of the epididymis. This is due to the fact that these various elements are not available in sufficient quantities. Due to interrupted sperm maturation, quality of sperm decreased. On the other hand, an increase in sperm maturation process will produce an increase in the number of live sperm cells which means an increase viability of spermatozoa (Misro M. AR M., Ganguly Das P. 2008).

Moreover, since red ginger also contains phenolic groups, the improvement of the quality of sperm is not only influenced by testosterone but also by the nature of the phenolic antioxidant of red ginger which acts as a free radical scavenger. However it requires further research.

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## CONCLUSION

It can be concluded that the infusion of red ginger (*Zingiber officinale* var. *Amarum*), especially at the dose of 20% affects testosterone and LH levels. However, red ginger at a dose of 40% increases testosterone levels and decreases levels of LH, due to the feedback effect of testosterone on the anterior pituitary.

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